R E M A R K S

The Advisory Action dated December 2, 2002 presents the examination of claims 1-11, 13-20, 23, and 26-32. Claims 11, 31, and 32 are canceled. Claims 1, 5, 7, 8, 10, 16, 18, 19, and 27 are amended. Upon entry of this Preliminary Amendment, claims 1-10, 13-20, 23, and 26-30 will be pending. No new matter is inserted into the application.

Interview

Applicants' representative thanks the Examiner for the helpful interview held at the United States Patent and Trademark Office on January 16, 2003.

New rejection under 35 U.S.C. § 112, second paragraph

In the Advisory Action, the Examiner rejects claims 1 and 16 under 35 U.S.C. § 112, second paragraph. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Claim 1

Specifically, the Examiner rejects claim 1 for allegedly being indefinite and for allegedly lacking support for the phrase "in the absence of any inhibitor or protein in the background." Said phrase is deleted from the claim. Thus, the rejection is overcome. Applicants replace the rejected phrase with "wherein

selection and use of the insulator sequence does not require prior knowledge of any inhibitor protein or any other regulatory component of the lethal gene." Support for this phrase is found on page 24, lines 11-13.

All of the prior art cited by the Examiner (e.g., Mariani et al., Williams et al., and Matthews et al.) teach production of male sterile plants using the *barnase* gene and the expression of an inhibitor protein of barnase, such as barstar, in tissues other than tapetum. A barstar-barnase complex is formed and the lethal effects of barnase are negated in all other tissues except for tapetum. Thus, all of these references use a barstar-barnase combination to produce normal and healthy male sterile plants. Accordingly, the teachings of Mariani et al., Williams et al., and Matthews et al. can only be used in situations where the lethal gene and the inhibitor protein-producing gene are known and produced as a combination by the system (plant) in question.

As opposed to this approach, the present invention employs a strategy which does not require any prior knowledge of any counter-protein or its production by the system. This feature of the present invention is recited in the instant claim 1. Further, this point is explained fully on page 6, wherein it is pointed out that the prior art strategy would only be effective in situations where a corresponding inhibitor protein for the male sterility gene is known. In other words, if the corresponding inhibitor protein is not present, then male sterility cannot be achieved.

In contrast to these prior art systems, the present invention creates male sterility without the prior knowledge of any co-regulatory gene or other functional component(s).

For these reasons, Applicants respectfully submit that the phrase "wherein selection and use of the insulator sequence does not require prior knowledge of any inhibitor protein or any other regulatory component of the lethal gene" is fully supported by the specification and reiterated in various ways such that the skilled artisan would not have any difficulty in understanding the invention.

Claim 16

The Examiner rejects claim 16 for lack of antecedent basis for the limitation of "the marker gene containing T1 progeny" in part (ix). Section (ix) of claim 16 is amended to recite "the T1 progeny containing the selectable marker gene." Antecedent basis for "the selectable marker gene" in found in section (i)(b) of claim 16. Thus, the instant rejection is overcome.

Rejections overcome

In the Advisory Action, the Examiner states that the Reply after Final filed on November 7, 2002 would have overcome the rejection under 35 U.S.C. § 102(e) over Williams '433 (USP 5,977,433) and the rejection under 35 U.S.C. § 102(b) over Chang '042 (USP 5,610,042). The Examiner also states that the

rejections under 35 U.S.C. § 103 would thus also be overcome. As the Request for Continued Examination filed on January 7, 2003 requested entry of the Reply after Final, the Examiner is respectfully requested to formally withdraw the rejections on the record.

Maintained rejection under 35 U.S.C. § 112, first paragraph

Enablement

The Examiner maintains the rejection of claims 1-11, 13-20, 23, and 27-31 under 35 U.S.C. § 112, first paragraph, for allegedly containing subject matter not enabled by the specification. Claims 11, 31, and 32 are canceled, thus rendering rejection thereof moot. Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Specifically, the Examiner asserts that the parent claims are not limited to insulator sequences that do not encode any functional protein. The Examiner also states that the claims should be limited to recite "tapetum" rather than "specific tissue(s)." In response to the Examiner's remarks, Applicants place the limitations of claim 11 into claim 1, and the limitations of claim 31 into claim 16. Claims 11 and 31 are canceled accordingly. Further, the claims are amended to recite

"tapetum" rather than "tissue" or "specific tissue(s)." Thus, the instant rejection is overcome.

Finally, the Examiner stated during the interview that she would like more information on the coding sequences of *topoisomerase* gene and *acetolactate synthase* gene recited in claims 10 and 30. The specification on page 19 states that the insulator sequence components comprise partial coding regions of *topoisomerase* gene and *acetolactate synthase* gene. Applicants offer the following comments.

The present invention uses a sequence having the properties set out in claim 1 as an insulator sequence. The illustrative example is Example 1 set out on pages 18-19 of the specification, which uses *topoisomerase* from pea and *acetolactate synthase* from *Arabidopsis*. The sequences (*topoisomerase* from pea and *acetolactate synthase* from *Arabidopsis*) are available in the EMBL database at accession No. Y14558 and X51514. These genes were cloned at NdeI-BglII sites and NcoI-XbaI sites. As such, the actual portions used may not need to be defined since any region out of these genes can be used. The only condition to be satisfied is that the sequence chosen must possess the properties described in claim 1.

Portions of the said sequences are selected, mobilized into a suitable transformation vector (as described in the specification) using convenient restriction sites, the selection of which is also well known to any person skilled in the art, and

used as insulators. For the Examiner's information, the actual portions of the (coding) sequences of the above-genes that were taken for construction of the Insulator sequence are as below:

topoisomerase I - nucleotide positions 183-2851

acetolactate synthase - nucleotide positions 313-2315

The length of the Insulator described in the present invention is about 5kb of which the contribution from partial coding sequences of *topoisomerase* I and *acetolactate synthase* gene is about 4.7kb. The final length of about 5kb is achieved by the incorporation of vector sequences containing various restriction sites that are normally introduced during various intermediate sub-cloning procedures.

Written Description

The Examiner also rejects claims 1-11, 13-20, 23, and 27-31 under 35 U.S.C. § 112, first paragraph, for allegedly not being described in the specification. Claims 11, 31, and 32 are canceled, thus rendering rejection thereof moot. Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

The Examiner states that the rejection would be overcome if the subject matter of claim 11 is inserted into claim 1 and the subject matter of claim 31 is inserted into claim 16. In response to the Examiner's remarks, Applicants place the

limitations of claim 11 into claim 1, and the limitations of claim 31 into claim 16. Claims 11 and 31 are canceled accordingly. Thus, the instant rejection is overcome.

Attached Papers

The Examiner states that the papers attached to the Reply after Final filed on November 7, 2002 were not sent. Applicants point out that the papers were sent to the USPTO as evidenced by the attached postcard receipt. In any event, Applicants attach hereto the papers for the Examiner's review.

Claim Objections

The Examiner objects to claims 1, 10, and 16. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Claim 1

The Examiner suggests inserting a comma after "elements" in claim 1, part (iii), line 3. Applicants amend claim 1 accordingly. Thus, the instant objection is overcome.

Claim 10

The Examiner states that claim 10 should recite "acid acetolactate synthase gene." Applicants respectfully disagree. During the interview, the Examiner stated that she had previously insisted on the recitation of "acid acetolactate synthase gene"

because this phrase is used in the specification. However, Applicants point out that "acid acetolactate synthase gene" is not recited in the specification. Rather, only the correct phrase "acetolactate synthase gene" is recited in the specification, such as on page 11, line 10. Applicants attach hereto two articles (Li et al. and Mazur et al.) evidencing that the correct terminology is "acetolactate synthase gene." The Examiner is respectfully requested to withdraw the claim objection.

The Examiner states that the limitation of "5kb in length" should be deleted from claim 10 since it is already recited in parent claim 1. Applicants amend claim 10 accordingly. Thus, the instant objection is overcome.

Claim 16

The Examiner suggests inserting a comma after "elements" in claim 16, part (c), line 3. Applicants amend claim 16 accordingly. Thus, the instant objection is overcome.

Conclusion

In view of the above remarks and/or amendments, Applicants respectfully submit that the claims are in condition for allowance. A Notice to such effect is earnestly solicited.

If the Examiner has any questions regarding the above, the Examiner is respectfully requested to contact Kristi L. Rupert,

Ph.D. (Reg. No. 45,702) at 703-205-8000 in the Washington D.C. area.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____
Raymond C. Stewart
Reg. No. 21,066
P. O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

RCS/KLR

Attachments: **Version With Markings To Show Changes Made**
Postcard receipt

Marjori Matzke, Antonius J.M. Matzke and Ortrun Mittelstein Scheid, 1994. "Inactivation of Repeated Genes - DNA-DNA Interaction?" *In* Homologous Recombination and Gene Silencing in Plants-, ed. Jerzy Paszkowski, pp 271-307.

Marjori A. Matzke and Antonius J.M. Matzke, 1995. "How and Why do Plants Inactivate Homologous (Trans)genes?- Plant Physiol. 107:679-685.

F. De Carvalho Niebel, P. Frendo, D. Inze, M. Cornelissen and M. Van Montagu, 1995. "Co-suppression of β-1,3-glucanase genes in *Nicotiana tabacum*" *In* Gene Silencing in higher plants and related phenomena in other eukaryotes-ed. P.Meyer, pp 91-103.

Angenent GC, Franken J, Busscher M, Colombo L, van Tunen AJ (1993) Petal and stamen formation in petunia is regulated by the homeotic gene fbp1. Plant J. 4: 101-112.

Angenent GC, Franken J, Busscher M, Weiss D, van Tunen AJ (1994) Co-suppression of the petunia

homeotic gene fbp2 affects the identity of the generative meristem. Plant J. 5: 33-34.

Mazur BJ, Chui C, Smith JK (1987) Isolation and characterization of plant genes coding for acetolactate synthase, the target enzyme for two classes of herbicides. Plant Physiol. 85:1110-1117.

Li R, McFerson JR, Kresovich S (2000) Genetic variation of the acetolactate synthase gene among cultivated *Brassica* species and its use in discrimination accessions of *B. napus* L. International Plant Genetic Resources Institute Newsletter, July 7, 2000.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claims 11, 31, and 32 are canceled.

The pending claims have been amended as follows:

1. (Three Times Amended) An insulator construct for controlling leaky expression of a lethal gene from enhancing functions of a strong constitutive promoter present in the said insulator construct following integration into the genome of a plant, said insulator construct comprising:

i) first transcription unit comprising a lethal gene under transcriptional control of a tapetum [tissue] specific promoter for targeted expression in tapetum [specific tissue(s)] and fused to a suitable transcription termination signal, comprising a polyadenylation signal,

ii) second transcription unit comprising a selectable marker gene under transcriptional control of a strong constitutive promoter and fused to a suitable transcription termination signal, comprising a polyadenylation signal, and

iii) an insulator sequence which is about 5kb in length, and which does not comprise transcriptional or other regulatory or enhancer elements, placed between the first and second transcription units so as to isolate the first transcription unit from enhancing influences of the constitutively expressing promoter in the second transcription unit,

wherein selection and use of the insulator sequence does not require prior knowledge of any inhibitor protein or any other regulatory component of the lethal gene, and [the insulator sequence functions in the absence of any inhibitor or protein in the background]

wherein the insulator sequence has the following properties:

(a) the insulator sequence does not encode any regulatory components or possess any enhancer elements or sequences that may influence the expression of neighboring genes;

(b) the insulator sequence has a GC content which is in consonance with transcriptionally active regions of a host genome;

(c) the insulator sequence does not produce any functional RNA or protein; and

(d) the insulator sequence does not bear strict homology with any component of the host genome in order to avoid induction of homology dependent gene silencing.

5. (Twice Amended) A construct as claimed in claim 1 wherein the tapetum [tissue] specific promoter of first transcription unit is selected from the group consisting of TA29, A9, A3, tap1, and bcp1[, and napin].

7. (Twice Amended) The construct as claimed in claim 1 wherein the selectable marker gene is selected from the group of herbicide resistance-conferring genes consisting of *bar* gene, *ALS* gene, and *tfdA* gene, or from the group of antibiotic resistance-conferring genes consisting of *nptII* gene, *hpt* gene and *aadA* gene.

8. (Twice Amended) The construct as claimed in claim 1 wherein the selectable marker gene is *bar* gene.

10. (Three Times Amended) The construct as claimed in claim 1 wherein the insulator sequence [is about 5kb in length and] comprises coding sequences of *topoisomerase* gene from pea and *acetolactate synthase* gene from *Arabidopsis*.

16. (Three Times Amended) A method to obtain male-sterile plants in *Brassica juncea*, said method comprising the steps of:

i) transforming the nuclear genome of plant cells with a foreign DNA comprising:

a) a first transcription unit comprising a lethal gene under transcriptional control of a tapetum [tissue] specific promoter for targeted expression in tapetum

[specific tissue(s)] and fused to a suitable transcription termination signal, comprising a polyadenylation signal,

b) a second transcriptional unit comprising a selectable marker gene under transcriptional control of a strong constitutive promoter and fused to a suitable transcription termination signal, comprising a polyadenylation signal, and

c) an insulator sequence which is about 5kb in length, and which does not comprise transcriptional or other regulatory or enhancer elements, placed between the first and second transcription units, so as to isolate the first transcription unit from enhancing influences of the constitutively expressing promoter in the second transcription unit,

wherein the insulator sequence comprises the following properties:

(a) the insulator sequence does not encode any regulatory components or possess any enhancer elements or sequences that may influence the expression of neighboring genes;

(b) the insulator sequence has a GC content which is in consonance with transcriptionally active regions of a host genome;

(c) the insulator sequence does not produce any functional RNA or protein; and

(d) the insulator sequence does not bear strict homology with any component of the host genome in order to avoid induction of homology dependent gene silencing;

ii) regenerating plants from said transformed plant cells,

iii) identifying male sterile transgenic plants by the absence of pollen production and by their failure to set seed on selfing,

iv) obtaining, at a high frequency, male sterile plants with normal vegetative morphology and normal female fertility,

v) identifying single copy male sterile lines by Southern hybridization,

vi) back-crossing male sterile plants with untransformed parent to obtain T1 seeds,

vii) obtaining male sterile plants with normal T1 seed germination frequencies,

viii) obtaining normal segregation ratio of the selectable marker gene among T1 progeny of single copy male sterile plants identified,

ix) transferring the [marker gene containing] T1 progeny containing the selectable marker gene to the field, and

x) identifying the male sterile phenotype among all T1 progeny exhibiting [marker] resistance to the selectable marker.

18. (Twice Amended) A methcd as claimed in claim 16 wherein the tapetum [tissue] specific promoter is TA29.

19. (Twice Amended) A method as claimed in claim 16 wherein the selectable marker gene is *bar* gene.

27. (Three Times Amended) A method as claimed in claim 16 wherein germinated T1 seedlings obtained from backcrossed seeds are tested for segregation of the selectable marker gene by transferring them onto selective media.